

PLUME

Questions

1) Are contaminant-degrading microorganisms present?

Aerobic

Anaerobic

Who are they?

Techniques/Answers

Incubation & Culture Studies - sequencing of isolates (~22)	UAF
SIP (BioTraps & Lab Microcosms)	FHR & UAF
DNA-SIP - 16s rRNA gene sequencing	UAF

Notes: There is potential to develop PCR primers & oligonucleotide probes specific for sulfolane degraders and for detection and long-term monitoring of sulfolane degrader populations. However, this is currently out-of-scope with the UAF RSA.

Timeline:

UAF: Incubations ongoing – sequencing information by Oct 2012; DNA-SIP by May 2013

FHR: BioTrap SIP data by Oct/Nov 2012

Answer:

UAF: Twenty-two sulfolane-degrading isolates have been enriched from groundwater, an air-sparge monitoring well, and spent GAC media. The isolates were identified using 16S rRNA gene sequencing. Isolates from the plume (MW110 and MW130) were members of the genera *Variovorax*, *Pseudomonas* and *Luteibacter*. The air sparge monitoring wells (AS4 and AS5) also yielded isolates from the genera *Variovorax*, *Pseudomonas* and *Luteibacter*, as well as *Flavobacterium*, *Pedobacter* and *Stenotrophomonas* strains. Spent filtration medium from one point of entry (POE) unit yielded *Flavobacterium*, *Brevundimonas* and *Pseudomonas* isolates. *Pseudomonas* and *Variovorax* strains

have previously been reported to degrade sulfolane, and Sphingomonas is known to degrade dibenzothiophene.

These isolates have been cultured on agar plates containing sulfolane as the sole carbon source. There is a small chance that the cultures grow at the expense of agar or other carbon sources present in trace levels. Experiments are now underway to verify that these isolates grow and degrade sulfolane in liquid culture, which would provide definitive evidence that they degrade sulfolane.

A molecular marker (T-RF) matching one sulfolane-degrading isolate was found in almost all of the groundwater samples but was missing from the upgradient sample

2) Are contaminant-degrading microorganisms active/degrading sulfolane? (in-situ)

- a. (Is biodegradation occurring?)
- b. By what metabolic pathways?

Techniques/Answers

BioTraps	FHR
BioTrap 13C-SIP	FHR
CSIA	FHR
Incubation & Culture & SIP mass balance & intermediate analysis	UAF
16s rRNA sequencing	develop PCR primers & oligonucleotide probes

Note: Development of PCR primers and oligonucleotide probes may allow use of a qPCR assay for long term monitoring of presence, abundance, and activity of sulfolane degraders within the plume. However, this is currently out-of-scope with the UAF RSA.

Timeline:

UAF: Incubations ongoing – sequencing information by Oct 2012; DNA-SIP by May 2013

FHR: BioTrap SIP and CSIA data by Oct/Nov 2012

3) What is the rate of biodegradation?

Techniques/Answers

Culture studies	-	model first-order degradation reactions	UAF
Incubation studies	-	model first-order degradation reactions	UAF
BioTrap 13C-SIP	-	relative mass balance	FHR
Lab 13C-SIP	-	mass balance	UAF
CSIA & GW isotopic enrichment studies - enrichment factor - Rayleigh Equation			UAF/FHR

Note: CSIA and GW isotopic enrichment studies can be performed using ¹³C-labelled sulfolane in groundwater incubations, with analysis by Uni. Oklahoma (CSIA) to determine a site-specific enrichment factor, which can be used in the Rayleigh Equation to estimate a rate of biotransformation.

UAF/FHR

Timeline:

UAF: May 2013

FHR: CSIA data by Oct/Nov 2012

a) What is rate limiting?

Techniques/Answers

Incubation studies - aerobic/anaerobic incubations with/without nutrient additions, multivariate analysis in relation to geochemistry and microbial communities in field	UAF
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May 2013—followup studies in summer? Multivariate analyses fall 2013

b) Can sulfolane biodegradation be enhanced?

Techniques/Answers

Incubation studies - aerobic/anaerobic incubations, nutrient additions, bioaugmentation (e.g., <i>Variovorax</i> or <i>Rhodococcus</i> spp.)	UAF
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Note: bioaugmentation studies, while potentially useful, are currently out-of-scope with the UAF RSA. However, nutrient additions are planned for incubation studies.

c) Does toxicity occur?

i) From sulfolane?

ii) From intermediates?

Techniques/Answers

Incubation studies & MicroTox	UAF
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Timeline:

UAF: Dec 2013 – May 2014

4) Does the presence of dissolved-phase LNAPL affect sulfolane biodegradation?

Techniques/Answers

Community analysis of T-RFLP data (include LNAPL presence & BTEX/sulfolane conc data)	UAF
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Timeline:

UAF: Dec 2013

Answer:

UAF: (preliminary) MW130 had sulfolane + petroleum and contained several unique T-RFs and some that increased in relative abundance when compared to wells without petroleum

5) Are degradation intermediates being produced?

Techniques/Answers

Incubation & Culture studies & intermediate analysis via GC/MS	UAF
BioTrap SIP & Lab SIP mass balance & intermediate analysis via GC/MS & GCIRMS	FHR/UAF

Note: intermediate analysis from GC/MS & GCIRMS (SIP experiments) may be inconclusive

Timeline:

UAF: May 2013 (preliminary),

FHR: BioTrap SIP and CSIA data by Oct/Nov 2012

6) Are intermediates affecting biodegradation?

Techniques/Answers

Incubation & Culture studies & MicroTox & intermediate analysis via LC/MS	UAF
BioTrap SIP & Lab SIP mass balance & intermediate analysis GC/MS & GCIRMS	FHR/UAF

Note: intermediate analysis from GC/MS & GCIRMS (SIP experiments) may be inconclusive. If intermediates are detected (e.g., by HPLC/MS), detection in the field may help explain differences in biodegradation activity within the plume.

Timeline:

UAF: Dec 2013 for microtox, preliminary data from incubations May 2013

FHR: BioTrap SIP and CSIA data by Oct/Nov 2012

7) Is biodegradation likely to continue?

Techniques/Answers

Long-term monitoring via qPCR, FISH, CSIA, contaminant trend analysis, geochemistry	FHR/UAF
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Note: : Development of PCR primers and oligonucleotide probes may allow use of a qPCR assay for long term monitoring of presence, abundance, and activity of sulfolane degraders within the plume. However, this is currently out-of-scope with the UAF RSA.

Timeline:

UAF: To be determined

FHR: Update contaminant trend analysis in quarterly report

8) Are other attenuation mechanisms occurring?

Techniques/Answers

Sterile controls	UAF
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a). What other attenuation mechanisms are occurring?

Techniques/Answers

Incubation studies with replicates & sterile controls	UAF
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Note: Some overlap with previous bench-scale studies by Barr Engineering will occur during laboratory incubations. Replicates will be employed, and sterile controls will be used to discriminate between abiotic and biological reactions.

Timeline:

UAF: Included in incubation studies—May 2013

Questions

1) Does aeration encourage biodegradation?

Techniques/Answers

Incubation studies & intermediate analysis
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UAF

Note: Intermediate analysis via HPLC/MS is pending; third party laboratory analysis may be pursued.

2) Does aeration encourage abiotic degradation?

Techniques/Answers

Incubation studies & intermediate analysis (sterile controls)

UAF

Timeline:

UAF: May 2013—addressed through incubations with samples not necessarily from air sparge system, but simulating it

3) Does aeration affect the microbial community?

Techniques/Answers

T-RFLP, sequencing & comparison with non-sparged areas
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UAF

Timeline:

UAF: Dec. 2013 concurrent with multivariate analyses

Answer: UAF: T-RF profiles from AS and are more similar to each other than they are to the other samples. The T-RF of sulfolane-degrading isolates makes up a larger proportion of the microbial community in AS samples than MW samples.

Caveat: different organisms can produce the same T-RF peak. DNA sequencing should be done to verify

4) Does aeration stimulate a different community of sulfolane degraders?

Techniques/Answers

SIP (lab)

UAF

Timeline:

UAF: Possibly May 2013, likely August

5) Does aeration produce intermediates, and if so, are they potentially toxic?

Techniques/Answers

Chemical analyses of intermediates/products produced by sulfolane degrading cultures, MicroTox assays of products	UAF
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Timeline:

UAF: Dec 2013—May 2014

6) Is degradation likely to continue?

Techniques/Answers

Long-term monitoring for contaminant trend analysis & geochemistry	FHR
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Timeline:

FHR: Continuing updates in quarterly reports

Additional considerations: scaling information to *in situ* dynamic conditions within the aquifer

How do we capture changing redox conditions (anaerobic to aerobic or microaerophilic conditions when the water table rises) in determinations of degradation rates?

Techniques/Answers

Alternating the sealing and re-opening of incubation flasks; possible occasional air sparge	UAF
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Possible start date of May 2013, depending on results from incubations

Will the magnitude of changing groundwater conditions on the sulfolane plume be known, and how will it affect sulfolane degradation?

Will that be captured in a “time to cleanup” projection?